Endocrine and metabolic consequences of preterm birth during early childhood

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Chapter 3

Urine gonadotropin and estradiol levels in female very-low-birth-weight infants

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ABSTRACT

Background

The postnatal activation of the hypothalamic-pituitary-gonadal axis is more exaggerated in preterm than in full-term born infants and may be important for future reproductive function.

Aim

The objective of this study was to investigate the postnatal activation of the hypothalamic-pituitary-gonadal axis in female very-low-birth-weight infants.

Study design

We performed serial measurements of gonadotropin and estradiol levels in urine samples of female very-low-birth-weight infants collected at 1 and 4 weeks postnatal age, at 32 weeks postmenstrual age, at expected date of delivery and at the corrected age of 3 and 6 months.

Subjects

Twenty-two very-low-birth-weight infants (gestational age 25.4-30.1 weeks), participating in the Neonatal Insulin Replacement Therapy in Europe trial, were included in this study.

Outcome measures

Gonadotropin and estradiol levels were measured in serial urine samples.

Results

Longitudinal analysis shows that after birth FSH and LH levels increase until 32 weeks postmenstrual age (4 weeks postnatal age) and then decrease until 3 months corrected age (26 weeks postnatal age). Estradiol levels decrease from 28 weeks postmenstrual age (1 week postnatal age) until 6 months corrected age (39 weeks postnatal age).

Conclusions

Serial urine sampling for measurement of gonadotropin and estradiol levels provides an accurate description of the postnatal activation of the hypothalamic-pituitary-gonadal axis in very-low-birth-weight girls. Levels of FSH and LH peak at a mean postmenstrual age of 32 weeks (postnatal age of 4 weeks), whereas estradiol levels are highest shortly after birth.

INTRODUCTION

The postnatal activation of the hypothalamic-pituitary-gonadal axis that is observed during the first months of life, is considered as an important phase in the maturation of the gonads and may play a significant role in the development of reproductive function. However, the mechanisms that underlie this activation and the exact importance for fertility are not well understood yet.

Several studies describe this postnatal activation in term born infants (1-6). In preterm born infants, the postnatal activation of the hypothalamic-pituitary-gonadal axis seems to be more exaggerated than in full-term born infants (7-11). Data about the postnatal activity of the hypothalamic-pituitary-gonadal axis in preterm infants born at a gestational age less than 30 weeks are limited.

Most of the studies in both term and preterm born infants have a cross-sectional design and report serum levels of gonadotropins and sex steroids. Serial serum samples give accurate information about the pattern of hormone secretion in individuals. However, serial blood sampling in infants also has major disadvantages, including the pain caused by the puncture and the risk of iatrogenic anemia, especially in very-low-birth-weight (VLBW) infants, and is qualified as a burden. These factors limit the number of serial blood samples that can be drawn for research purposes from one infant in a period of time. Urine sampling does not have these disadvantages and both gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) can be reliably measured in urine samples (12, 13).

It was hypothesized that serial gonadotropin levels in combination with serial estradiol levels measured in urine during the first postnatal months, can be used to describe the postnatal activation of the hypothalamic-pituitary-ovarian axis in female VLBW infants. Therefore the aim of the present study was to investigate gonadotropin and estradiol secretion in serial urine samples of female VLBW infants from birth to 9 months of age.

METHODS

Study population

The subjects were part of the Neonatal Insulin Replacement Therapy in Europe (NIRTURE) trial. This was an international multicenter randomized controlled trial investigating the role of early insulin therapy in VLBW infants (14). After informed parental consent was obtained, VLBW infants younger than 24 hours of age were randomized to receive continuous intravenous infusion of insulin for the first 7 days of life or standard neonatal care with insulin treatment only in case of hyperglycemia. Exclusion criteria included maternal diabetes and major congenital anomalies.

The 22 female infants participating in the NIRTURE trial in our neonatal intensive care unit were included in the present study. They had a mean gestational age of 27.6 weeks (range 25.4-30.1 weeks) and a mean birth weight of 998 g (range 540-1415 g). Ten infants were assigned to the early-insulin group and 12 infants received standard neonatal care. After discharge, all patients were followed in the outpatient clinic. Approval from the local ethics committee was obtained.

Urine samples

Urine samples were collected in a pediatric urine collection pouch at 1 and 4 weeks postnatal age, at 32 weeks postmenstrual age, at expected date of delivery and at the corrected age of 3 and 6 months. Samples were stored at -20 ^{o}C until analysis.

FSH, LH and estradiol measurement

FSH and LH concentrations were measured by immunometric assays (Architect, Abbott Laboratories Diagnostics Division, Abbott Park, Illinois, USA). For FSH lower limit of quantitation is 0.11 IU/l, intra-assay coefficient of variation is 3% at 5.5 IU/l, 25 IU/l and 75 IU/l and inter-assay coefficients of variation are 6% at 5 IU/l and 5% at 18 IU/l. For LH lower limit of quantitation is 0.1 IU/l, intra-assay coefficient of variation is 3% at 5 IU/l, 40 IU/l and 75 IU/l and inter-assay coefficients of variation are 7% at 4 IU/l and 6% at 23 IU/l.

After hydrolysis with helix pomatia juice (Pall Biosepra, Cergy-Saint-Christophe, France) and extraction with diethyl ether, urinary estradiol concentration was measured by competitive immunoassay (Architect, Abbott Laboratories Diagnostics Division, Abbott Park, Illinois, USA), as also applied by Peper et al. (15). Intra-assay coefficients of variation are 9%, 3% and 4% at a level of 150, 1400 and 9000 pmol/l, respectively and inter-assay coefficient of variation is 10% for the whole range.

Gonadotropin and estradiol levels were corrected for creatinine levels. Creatinine concentrations were measured by the Jaffé method (Modular, Roche Diagnostics, Mannheim, Germany). Inter-assay coefficients of variation are 2.2% at 5.9 mmol/l and 1.7% at 12.5 mmol/l.

Statistical analysis

Statistical analyses were performed using the Statistical Package of Social Sciences software for Microsoft Windows version 17 (SPSS Inc., Chicago, Illinois, USA). At first FSH, LH and estradiol levels of infants treated with insulin were compared with hormone levels of infants receiving standard care. Because the distribution of hormone levels was skewed, a log transformation was performed before analysis. To distinguish the role of birth from that of maturation in the activation of the hypothalamic-pituitary-gonadal axis, FSH, LH and estradiol levels were analyzed in relation to postmenstrual age as well as to postnatal age. If the influence of birth itself is most important for the activation, peak hormone levels are expected to be found at comparable postnatal ages as in term

born infants; if, on the other hand, the degree of maturation comparable to term born infants is required for the activation of the hypothalamic-pituitary-gonadal axis, then peak hormone levels are expected to be found at comparable postmenstrual ages (ages corrected for prematurity). Longitudinal analyses were performed with generalized estimating equations, a method that takes into account that the repeated observations in one child are correlated. For gonadotropin levels below the limit of detection, a value of 0.01 U/l was used. P values < 0.05 were considered as significant.

RESULTS

From our study population of 22 female VLBW infants, a total of 94 urine samples were collected for determination of FSH, LH and estradiol levels.

Levels of FSH, LH and estradiol for postmenstrual age

The urine samples were divided into six age groups, based on the postmenstrual age on the day of collection of the sample. The details of the age groups with mean postmenstrual age and number of samples as well as the median levels of FSH, LH and estradiol are shown in table 1. Because hormone levels were not different between the early-insulin and standard care group, the groups were taken together for further analyses.

Longitudinal analysis showed that after birth FSH levels significantly increased from 28 weeks postmenstrual age to 32 weeks postmenstrual age. Thereafter FSH levels significantly decreased until 53 weeks postmenstrual age (corrected age of 3 months) (Table 1 and Figure 1). LH levels significantly increased from 30 weeks postmenstrual age to 32 weeks postmenstrual age. Thereafter LH levels significantly decreased until 53 weeks postmenstrual age (corrected age of 3 months) (Table 1 and Figure 1). Estradiol levels significantly decreased from 28 weeks postmenstrual age until 66 weeks postmenstrual age (corrected age of 6 months) (Table 1 and Figure 2).

Levels of FSH, LH and estradiol for postnatal age

For this analysis, urine samples were divided into five age groups, based on the postnatal age at the time of collection of the sample. Table 2 shows the details of these age groups with mean postnatal age, number of samples and median levels of FSH, LH and estradiol. Hormone levels were not different between the early-insulin and standard care group and the groups were taken together for further analyses.

Longitudinal analysis showed that after birth both FSH and LH levels significantly increased from 1 week to 4 weeks postnatal age. Thereafter FSH and LH levels significantly decreased until 26 weeks postnatal age (Table 2 and Figure 3). Estradiol levels significantly decreased from 1 week postnatal age until 39 weeks postnatal age (Table 2 and Figure 4).

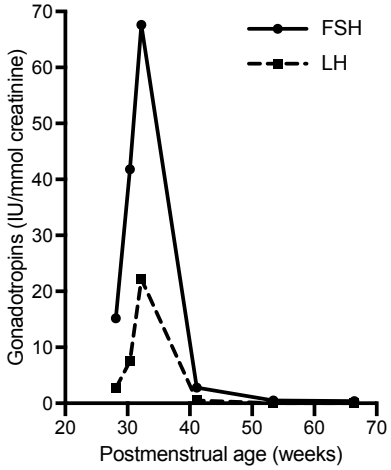

Figure 1. Median FSH and LH levels for samples collected from six age groups with increasing mean postmenstrual age.

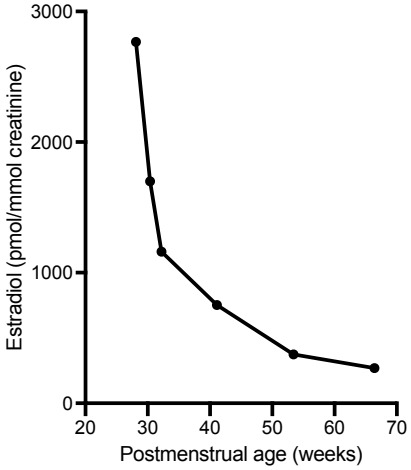

Figure 2. Median estradiol levels for samples collected from six age groups with increasing mean postmenstrual age.

Table 1. FSH, LH and estradiol levels for samples collected from six age groups based on postmenstrual age

Group		N	Postmenstrual age (weeks)	FSH (IU/mmol creatinine)	LH (IU/mmol creatinine)	Estradiol (pmol/mmol creatinine)
I	total	15	28.1 (26.4-29.1)	15.2 (0.7-86.0)	2.7 (0.01-30.8)	2767 (1343-12629)
	standard	6	28.3 (27.4-29.1)	8.6 (1.4-86.0)	0.9 (0.1-17.2)	2522 (1401-3820)
	insulin	9	27.9 (26.4-29.1)	18.4 (0.7-61.7)	6.7 (0.01-30.8)	3893 (1343-12629)
II	total	11	30.4 (29.4-31.1)	41.8 (2.5-258.2)	7.5 (0.01-49.3)	1700 (1053-4020)
	standard	7	30.6 (29.4-31.1)	23.0 (2.5-91.0)	6.7 (0.01-10.7)	1700 (1053-4020)
	insulin	4	30.0 (29.4-30.6)	93.0 (7.7-258.2)	16.4 (1.9-49.3)	1698 (1152-2165)
III	total	14	32.2 (31.6-33.6)	67.6 (25.1-183.5)	22.2 (0.9-65.4)	1160 (659-2873)
	standard	10	32.1 (31.6-33.4)	64.4 (25.1-183.5)	17.5 (0.9-65.4)	1268 (659-2855)
	insulin	4	32.5 (32.0-33.6)	98.8 (53.9-141.8)	23.6 (13.2-29.1)	955 (862-2873)
IV	total	21	41.1 (39.6-44.7)	2.8 (0.6-33.7)	0.5 (0.01-10.7)	752 (200-1952)
	standard	12	41.0 (39.6-42.1)	2.7 (0.6-20.7)	0.08 (0.01-10.7)	791 (200-1740)
	insulin	9	41.2 (40.0-44.7)	7.3 (0.8-33.7)	1.1 (0.07-4.3)	534 (292-1952)
V	total	20	53.4 (52.0-56.0)	0.5 (0.01-2.1)	0.01 (0.01-0.2)	374 (155-697)
	standard	11	53.5 (53.0-56.0)	0.3 (0.01-1.1)	0.01 (0.01-0.01)	348 (155-697)
	insulin	9	53.1 (52.0-54.0)	0.7 (0.01-2.1)	0.01 (0.01-0.2)	406 (240-676)
VI	total	13	66.4 (65.0-68.0)	0.4 (0.03-3.8)	0.01 (0.01-0.1)	269 (116-469)
	standard	9	66.4 (65.0-68.0)	0.4 (0.03-3.8)	0.01 (0.01-0.1)	265 (116-460)
	insulin	4	66.3 (66.0-67.0)	0.8 (0.4-2.2)	0.01 (0.01-0.01)	307 (220-469)

Hormone levels are expressed as median and range, postmenstrual age as mean and range. For each age group, results are given for all samples together, for samples of infants in the standard care group and for samples of infants in the early-insulin group. In all age groups, gonadotropin and estradiol levels did not differ significantly between the standard care and early-insulin group.

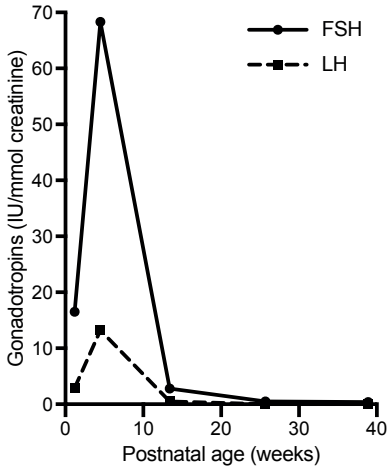

Figure 3. Median FSH and LH levels for samples collected from five age groups with increasing mean postnatal age.

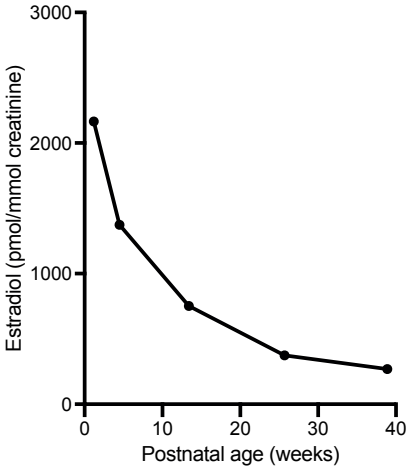

Figure 4. Median estradiol levels for samples collected from five age groups with increasing mean postnatal age.

Table 2. FSH, LH and estradiol levels for samples collected from five age groups based on postnatal age

Group		N	Postnatal age (weeks)	FSH (IU/mmol creatinine)	LH (IU/mmol creatinine)	Estradiol (pmol/mmol creatinine)
I	total	23	1.2 (1.0-3.6)	16.5 (0.7-86.0)	2.9 (0.01-30.8)	2165 (1053-12629)
	standard	13	1.4 (1.0-3.6)	15.2 (1.4-86.0)	2.9 (0.01-24.1)	1700 (1053-4020)
	insulin	10	1.0 (1.0-1.0)	17.5 (0.7-61.7)	4.7 (0.01-30.8)	3775 (1343-12629)
II	total	17	4.5 (4.0-6.6)	68.3 (7.7-258.2)	13.2 (0.9-65.4)	1374 (659-3210)
	standard	10	4.6 (4.0-5.7)	64.4 (7.7-183.5)	11.6 (0.9-65.4)	1592 (659-3210)
	insulin	7	4.4 (4.0-6.6)	129.3 (53.9-258.2)	24.4 (8.3-49.3)	1152 (862-2873)
III	total	21	13.4 (10.3-18.3)	2.8 (0.6-33.7)	0.5 (0.01-10.7)	752 (200-1952)
	standard	12	12.9 (10.3-15.6)	2.7 (0.6-20.7)	0.08 (0.01-10.7)	791 (200-1740)
	insulin	9	14.1 (10.6-18.3)	7.3 (0.8-33.7)	1.1 (0.07-4.3)	534 (292-1952)
IV	total	20	25.7 (23.4-28.3)	0.5 (0.01-2.1)	0.01 (0.01-0.2)	374 (155-697)
	standard	11	25.4 (23.6-27.7)	0.3 (0.01-1.1)	0.01 (0.01-0.01)	348 (155-697)
	insulin	9	26.0 (23.4-28.3)	0.7 (0.01-2.1)	0.01 (0.01-0.2)	406 (240-676)
V	total	13	38.9 (35.0-41.6)	0.4 (0.03-3.8)	0.01 (0.01-0.1)	269 (116-469)
	standard	9	38.7 (35.0-40.9)	0.4 (0.03-3.8)	0.01 (0.01-0.1)	265 (116-460)
	insulin	4	39.4 (37.9-41.6)	0.8 (0.4-2.2)	0.01 (0.01-0.01)	307 (220-469)

Hormone levels are expressed as median and range, postnatal age as mean and range. For each age group, results are given for all samples together, for samples of infants in the standard care group and for samples of infants in the early-insulin group. In all age groups, gonadotropin and estradiol levels did not differ significantly between the standard care and early-insulin group.

DISCUSSION

In the present study, we confirm postnatal activation of the pituitary-ovarian axis in preterm born female infants. Moreover, by serial measurement of gonadotropins and estradiol using urine samples we were able to get an accurate impression of the postnatal activation of the pituitary-gonadal axis in VLBW girls.

Previous studies in female term born infants indicated measurable serum gonadotropin levels with peak concentrations during the first months of life, followed by a decline to pre-pubertal levels during the first years of life: FSH peak values were measured in the first 3 months of life and levels stayed above those of older pre-pubertal children until 4 years of age; LH values were always lower than FSH values, reached maximum levels around 1 month of age and were in the normal pre-pubertal range after 4 months of age (2, 4, 5).

In contrast to gonadotropin levels, estradiol levels are high in cord serum followed by a rapid fall after birth due to disappearance of placental estrogens, reaching pre-pubertal levels 4 months after birth (6). A possible explanation for the postnatal pituitary-gonadal activation is that this fall in estradiol concentration causes the rise in gonadotropin concentrations by loss of inhibitory feedback. In turn, high levels of gonadotropins result in ovarian stimulation and production of estradiol. With maturation of the inhibitory feedback system by gonadal steroids, the gonadotropin concentrations decline again with increasing age (5, 6). The exact importance of this postnatal activation for normal development and function of the ovaries and future fertility is still unclear.

In preterm born female infants, serum gonadotropin levels are higher during the first 10 weeks of life compared to term born female infants, with FSH levels 10-20 times higher and LH levels 3-4 times higher (10). Peak FSH and LH levels are reached between 11 and 30 postnatal days and the peak is more marked and prolonged in preterm compared to term born female infants (9). To explain these differences between preterm and term born female infants, it was suggested that the higher gonadotropin levels in premature born girls were probably caused by the immature state of the hypothalamic-pituitary-gonadal axis, which could be less sensitive for negative feedback by the postnatal decline in estradiol levels than in term born infants (10). Measurement of estradiol levels in 3-month-old girls shows that preterm born girls also have higher estradiol levels than term born girls (16). The relevance of the exaggerated activation of the pituitary-ovarian axis in preterm girls for ovarian development and reproductive function is not yet known.

Cross-sectional data of serum gonadotropin levels during the first 6 weeks of life collected from extremely premature infants born between 24 and 29 weeks gestational age show very variable FSH and LH levels in female infants without any obvious peak

level during this period (17). As we used urine samples in the present study, we were not limited by the disadvantages of serial serum sampling.

Kuiri-Hanninen et al. (8, 11) recently reported gonadotropin levels in serial urine samples of full-term and preterm born infant boys and girls. In boys they demonstrated increased postnatal hypothalamic-pituitary-gonadal axis activation associated with faster testicular and penile growth in preterm compared to full-term born infants (8). In addition, they found that in term as well as preterm girls FSH levels were highest at 1 month of age and resulted in transient ovarian stimulation. As they observed a delay in ovarian folliculogenesis in preterm compared to full-term born girls, it was speculated that insufficient inhibitory feedback by ovarian inhibins and estrogens could cause the higher and more prolonged FSH peak in preterm girls (11). Kuiri-Hanninen et al. (11) studied preterm girls with a wide range of gestational ages (24.7 to 36.7 weeks) and birth weights (530 to 2720 g). We studied the hypothalamic-pituitary-gonadal axis using serial urine samples in preterm girls all born between 25.4 and 30.1 weeks with birth weights less than 1500 g.

In our study, both FSH and LH show a peak at a mean postmenstrual age of 32 weeks, corresponding to a mean postnatal age of 4 weeks. As peak hormone levels were measured at a comparable postnatal age as in previous studies in term born female infants (2, 4, 5), this suggests that birth itself plays a crucial role in the activation of the hypothalamic-pituitary-gonadal axis. At 3 and 6 months corrected age FSH levels are low, but still measurable in most infants, while at that time LH levels are immeasurable in most infants. This is in accordance with the results of Winter et al. (5), who described persistence of higher FSH levels until 4 years of age. The absence of an obvious peak in gonadotropin concentrations in an earlier study of Greaves and Hunt et al. (17) in extremely premature female infants may have been caused by the cross-sectional design of that study. Cross-sectional studies are more limited in detecting patterns of hormone secretion than longitudinal studies.

As far as we know estradiol levels have not been measured serially in preterm born girls in previous studies. Estradiol levels are highest in the youngest age group (mean postmenstrual age of 28 weeks) and decrease with increasing age. The decrease after birth is caused by the disappearance of placental estrogens. Peak gonadotropin levels are preceded by a decrease in estradiol levels, supporting the hypothesis that the rise in gonadotropin concentrations is caused by a decrease of inhibitory feedback by estradiol. Kuiri-Hanninen et al. (11) showed ovarian stimulation following the postnatal FSH peak, so it is probable that rising gonadotropin levels stimulate the ovarian production of estradiol. This stimulation is maximal around 4 weeks of age and subsequently decreases. Despite this ovarian stimulation estradiol levels in our study did not show a peak. It is possible that we did not find this peak because of insufficient data between days 7 and 28 or the small number of infants. On the other hand, Winter et al. (6) studied

postnatal estradiol levels in term born female infants and did not find a peak either; they suggested that the considerable variability in estradiol levels in female infants could be caused by growth and regression of ovarian follicles.

Limitations of our study are the small number of infants and the lack of data from term born female infants. The results have to be confirmed in a larger group of VLBW infants. To make a good comparison with the patterns and values of gonadotropins and estradiol in term born girls, a study should be performed with use of the same assays to measure levels of gonadotropins and estradiol in serial urine samples of term born female infants.

In conclusion, by using urine samples we were able to collect serial measurements of gonadotropin and estradiol levels in female VLBW infants without the burden of frequent blood sampling. This provides an accurate description of the postnatal activation of the hypothalamic-pituitary-gonadal axis in VLBW girls. Levels of FSH and LH peak at a mean postmenstrual age of 32 weeks (postnatal age of 4 weeks) and estradiol levels are highest shortly after birth.

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